Structural genomics of Mimivirus proteins

The *Acanthamoeba polyphaga mimivirus* NDK:

The complete sequence of the largest known double-stranded DNA virus, *Acanthamoeba polyphaga mimivirus*, was recently determined (Raoult *et al.,* 2005) and revealed numerous genes not expected to be found in a virus. A comprehensive structural and functional study of these gene products was initiated (Abergel *et al.*, 2005) both to better understand their role for the virus physiology and to get some clues on the origin of DNA viruses.

Among the proteins never identified before in a viral genome, *Acanthamoeba polyphaga mimivirus* includes a Nucleoside Diphosphate Kinase (NDK). The NDKs are required for the synthesis of nucleoside triphosphates (NTP) other than ATP (Parks & Agarwal, 1973) (EC: 2.7.4.6). They are non specific enzymes active on both purine and pyrimidine, ribo- or desoxyribonucleotides and they can provide NTPs or dNTPs for nucleic acid synthesis, CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization.

The sequence analysis of the *Acanthamoeba polyphaga mimivirus* nucleoside diphosphate kinase (NDK) highlights a specific feature of the viral protein located at the substrate binding site. Enzymatic studies of this enzyme together with its structural analysis should provide some insights on the viral NDK specificity and its functional role in the context of the amoeba infection. We cloned and expressed the NDK using *Escherichia coli* expression system (Rosetta(DE3)pLysS) and 2 crystal forms of the purified recombinant protein have been produced. Crystal belongs to the cubic space group, $P2_13$ with cell dimensions of 99.425 (Jeudy *et al.*, 2005).

The crystal structure of the Mimivirus NDK has been solved using a data set collected on a MAR CCD camera at the European Synchrotron Radiation facility (ID29 beamline) at a wavelength of 0.97563 Å (Figure 1). There is two monomers per asymmetric unit related by a two fold non crystallographic axis and thus four biological entities per unit cell. The structure was solved by molecular replacement using the CaspR web-server (Claude *et al.*, 2004; http://igs-server.cnrs-mrs.fr/Caspr/index.cgi) and the 1K44 and 1NDL structures as references to generate the *Acanthamoeba polyphaga mimivirus* NDK models.

The activity of the purified recombinant enzyme has been verified and we are currently assaying its specificity. In the meantime, the structural analysis of the *Acanthamoeba*

polyphaga mimivirus NDK is performed and we use the BM30A beamline on crystals obtained by co-crystallizing the protein with various NDP. To date we were only able to locate the phosphate groups of the ligands the rest of the NDP structure being disordered. We are still currently trying to identify crystallization conditions at neutral pH in order to stabilize the ligand in the NDK active site. This study should provide a better understanding of the NDK molecular function and therefore of its functional role in the context of the *Acanthamoeba polyphaga* infection.

References:

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Figure 1:

Cartoon representation of the hexameric NDK biological unit.